GENERAL PRINCIPLES OF THE INITIATION OF REGENERATION OF THE BONE MARROW SUPPRESSED IN ANIMALS AFTER MASSIVE DOSES OF CYTOSTATIC AGENTS

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UDC 615.277.3.015:
[612.6.03:612.419

Experiments on guinea pigs and rats showed that after administration of many cytostatic agents in doses of LD_{25}-LD_{50}, toward the end of the period of devastation large hemohistioblasts of reticular nature appear in the bone marrow on the 6th-8th day, rapidly undergo differentiation, and turn into cells of the myeloid series. The beginning of regeneration of the myeloid series coincides in time with the end of the leukopenic phase and an increase in the blood white cell count. The beginning of regeneration of the erythroid series is connected with the direct transformation of reticular cells into erythro-normoblasts.

Investigation of regeneration in the depopulated bone marrow of animals can shed light on the principles governing the regeneration of the hematopoietic tissue and its course in patients during treatment and the compulsory withholding of cytostatic agents.

EXPERIMENTAL

Experiments were carried out on guinea pigs and rats (over 500 animals) in which changes in the blood and bone marrow picture were studied after administration of a single dose of various cytostatic agents (cyclophosphamide, imiphos, aziprin, asaline [N-(N-acetylsarcolysyl)-valine], carcolysin [phenylalanine mustard], rubomycin, 6-mercaptopurine, 5-fluorouracil, and ftorafur) ranging from LD_{50} to LD_{25}. The blood and sternal bone marrow were investigated until the restoration of normal hematopoiesis. Films were stained with azure II-eosin, and at certain periods of the lesion they were also stained for lipids (the method of Sheenan and Storey), for peroxidaze (by the method of Sato and Sekia), and for polysaccharides (by the method of McManus and Hotchkiss). The normal number of myelokaryocytes in the bone marrow of healthy guinea pigs was found to be (in thousands) 1102 ± 44.2 ($\pm\sigma = 230$) and in rats 1253 ± 24 ($\pm\sigma = 176$) per mm^2 sternal marrow (Fig. 1). In order to analyze the changes in the myelograms the relative percentages of the individual groups of cells were converted into absolute numbers, so that the kinetics of the reticular cells and changes in the curves of the individual cell series could be identified.

EXPERIMENTAL RESULTS

On the second day after administration of many of the cytostatics in large doses, the number of myelokaryocytes began to fall even before the development of leukopenia, and giant hemohistioblasts (up to 27-30 μ in diameter) and their derivatives – giant neutrophilic leukocytes – appeared in the bone marrow. It was shown previously that the "gigantism phenomenon" observed during the first 2-3 days can be regarded as a sign of imminent hypoplasia of the bone marrow [1-3].

Department of Pathophysiology and Hematological Laboratory, Central Research Laboratory, Tomsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR I. V. Toroptsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 11, pp. 96-99, November, 1972. Original article submitted April 7, 1972.

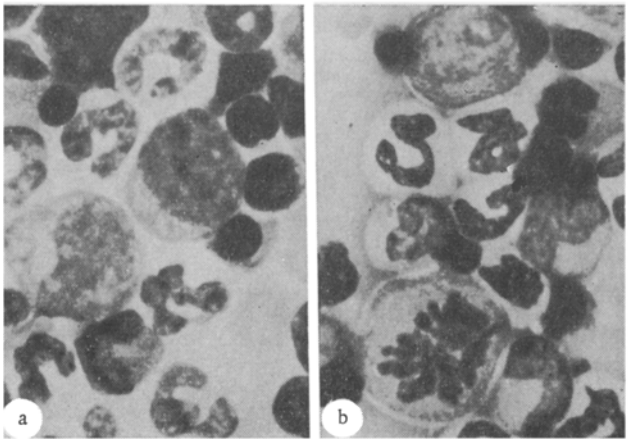

Fig. 1

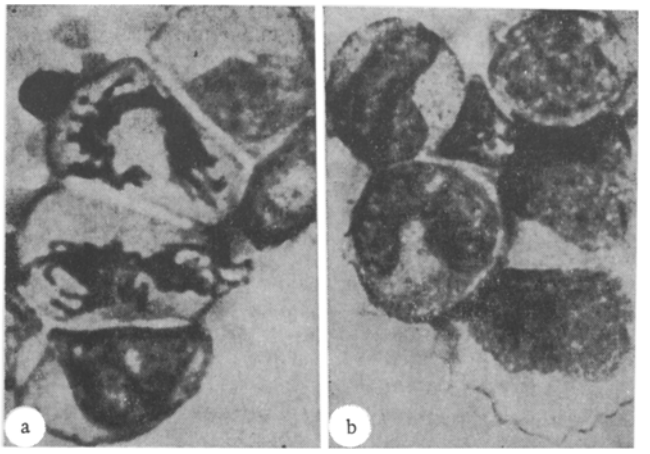

Fig. 2

Fig. 1. Bone marrow of healthy animals: a) guinea-pig marrow; two hemocytoblasts in the center; b) rat marrow: myeloblast in mitosis below the center, myelocyte above. 1400 ×.

Fig. 2. Bone marrow of rat on 6th day after injection of asaline in dose of 187 mg/kg. a) Hemohistioblasts, abnormal mitoses; b) another field, differentiation of hemohistioblasts into cells of myeloid series; two reticular cells on the right. 1400 ×.

Toward the end of the period of depopulation, corresponding to the 5th-8th day after administration of massive doses of the cytostatics, the number of myelokaryocytes fell to 200,000-100,000, and the bone marrow films contained only reticular cells or their bare nuclei, macrophages, and a certain number of lymphocytes. At the same time, variable numbers (12-35%) of large blast cells of unusual type for the aplastic state began to appear: the hemohistioblasts which the writers previously called monocytoid cells. These cells attained a diameter of up to 20-22 μ, i.e., they were larger than the hemocytoblasts and myeloblasts, whose mean diameter in healthy guinea pigs is $16.27 \pm 0.02\,\mu \pm \sigma = 0.02\,\mu$) and in healthy rats $16.3 \pm 0.11\,\mu$ ($\pm\sigma = 2.22$). The hemohistioblasts which appeared were distinguished by some degree of atypism, expressed not only by their larger size, but also by their reduced nucleocytoplasmic ratio, the polymorphism of their nucleus (round, oval, lobular), and also the considerable basophilia (pyroninophilia) of their cytoplasm and its freedom from granules. Mitotic figures were often found, most commonly abnormal metaphases (Fig. 2a). At the earliest periods lipids and peroxidase activity were absent and only traces of polysaccharides were present. Starting from the first day, however, from the moment of their appearance in some of the hemohistioblasts, sudanophilic and peroxidase granules began to appear in the perinuclear zones and PAS-positive material accumulated. Whereas the number of these cells initially was small (about 10%), after the second day up to 40-50% of the blast cells were cytochemically active. Rapid differentiation of the hemohistioblasts into myeloblasts was thus taking place. As differentiation continued (into pro-, myelo-, and metamyelocytes) the number of peroxidase and sudanophilic granules increased.

Morphological differentiation of the hemohistioblasts was observed parallel with the above changes; an oxyphilic perinuclear zone began to appear, areas of azurophilic and neutrophilic granules were found, the chromatin structure of the nucleus became coarser, and as it matured, promyelocytes and myelocytes and cells resembling metamyelocytes and polymorphonuclear cells were formed (Fig. 2b). Meanwhile, maturation of the cytoplasm was delayed and in many of the newly appearing giant forms of metamyelocytes and polymorphonuclear neutrophils a varied degree of basophilia of the cytoplasm was present. Quantitative analysis of the myelograms showed that 1-2 days from the time of appearance of the hemohistioblasts the curve of immature myeloid forms began to rise sharply, and this was almost immediately followed by a rise in the curve of polymorphonuclear neutrophils. At the same time the blood white cell count began to increase; many of these cells were enlarged and contained remnants of basophilic cytoplasm. The initial phase of recovery of myelopoiesis thus proceeded along the following lines: reticular cell → hemohistioblast → myeloblast → promyelocyte → myelocyte → metamyelocyte → mature granulocyte.

So far as the erythroid series is concerned, the beginning of its regeneration was observed less frequently before the appearance of the blast forms (experiments with cyclophosphamide), but more frequently

1-2 days after the hemohistioblasts, although the latter play no part in the formation of erythroid cells. The initial phenomena of erythropoiesis were connected with the direct transformation of reticular cells into erythro-normoblasts, and the latter to begin with could preserve their connection with the group of reticular cells and also the reticular tapering of their cytoplasm.

It can be concluded from the study of the dynamics of inhibition of hemotopoiesis in the bone marrow of guinea pigs and rats and the initial stages of the course of regeneration after administration of large doses of cytostatic agents that certain general principles can be detected which play a role in the regeneration of the myeloid series and which are manifested after the end of the period of depopulation. First, by the end of the first week or beginning of the second there is an increase in the absolute number of reticular cells, some of which are liberated from their syncytial connections, and giant blast cells (hemohistioblasts) are formed. The latter are able to rapidly differentiate into cells of the myeloid series. A comparative investigation of the dynamics of the blood and marrow pictures shows conclusively that in many cases myelopoiesis is effective, for the leukopenic phase ends and the curve of the blood white cell count begins to rise.

The writers suggest that the principles described above at the beginning of regeneration may also apply in patients during treatment with antitumor compounds if marked hypoplasia of the hematopoietic tissue develops. If this hypothesis is confirmed, it will have to be borne in mind that the large blast cells which may appear in the depopulated bone marrow of patients with hemoblastoses at the beginning of the recovery period must not be regarded as remnants of anaplastic cells of the leukemic clone.

LITERATURE CITED

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